# Effects of Microtubule Intersections on Binding of the Kinesin-5 Motor Cin8

Stephanie Dewald
Advisor: Dr. Melissa Gardner

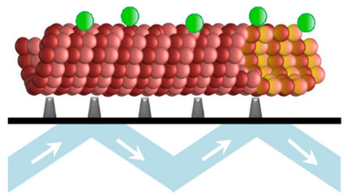
## BACKGROUND

- Mitosis is the process of cellular division and chromosome separation.
- Improper chromosome segregation is characteristic in cancer cells and can also lead to birth defects.
- Microtubules are cellular structures responsible for chromosome segregation
- Proper microtubule length regulation is needed for mitosis to occur correctly[1].
- Motor proteins can influence microtubules and "walk" along microtubules. This may cause growth or catastrophe (shortening) or the microtubule[2,3].
- The Kinesin-5 motor protein Cin8, a tetramer, has been shown to regulate length and positions of microtubules *in vivo*, but not *in vitro*[4].

## PURPOSE

- The primary purpose of this research is to determine if Cin8 localizes at the intersections of two microtubules.
- Another question looked at is if the amount of Cin8 localized at an intersection is dependent on the angle formed between the two microtubules.
- Localization of Cin8 to microtubule intersections could play a role in separating spindle poles and establishing a mitotic spindle.

## EXPERIMENTAL SETUP




## SNAPSHOTS

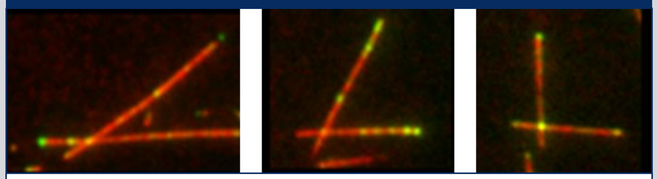


**Figure 1**: Examples of cropped microtubules (red) and Cin8 (green). From left to right, the intersecting microtubules show a low angle, medium angle, and high angle, respectively, in regard to the acute angle formed from the intersection of the microtubules.

## METHODS

- GMPCPP-tubulin seeds were attached to the bottom coverslip in the chamber with an anti-rhodamine antibody
- Cell lysates containing the protein Cin8 labeled with GFP were flowed into the chamber.
- Imaged by Total Internal Reflection Fluorescence (TIRF) microscopy. Microtubule seeds appear red and Cin8-GFP appears green
- Snapshots were taken and analyzed with ImageJ, and intersecting microtubules were cropped out (Figure 1). Cropped images were analyzed with MATLAB code written by Dr. Melissa Gardner. The basic set up of the code analysis is seen in Figure 2.
- The relationship between intersection angle of crossing microtubules and normalized Cin8-GFP localized at the intersection compared to the lattice of the microtubule was determined.

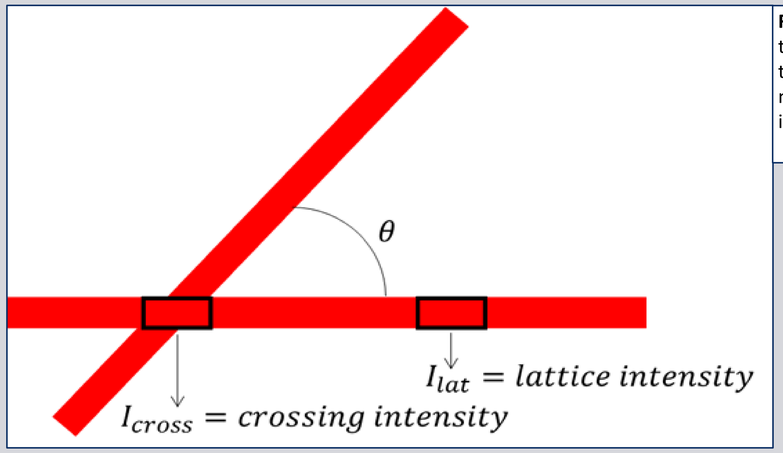
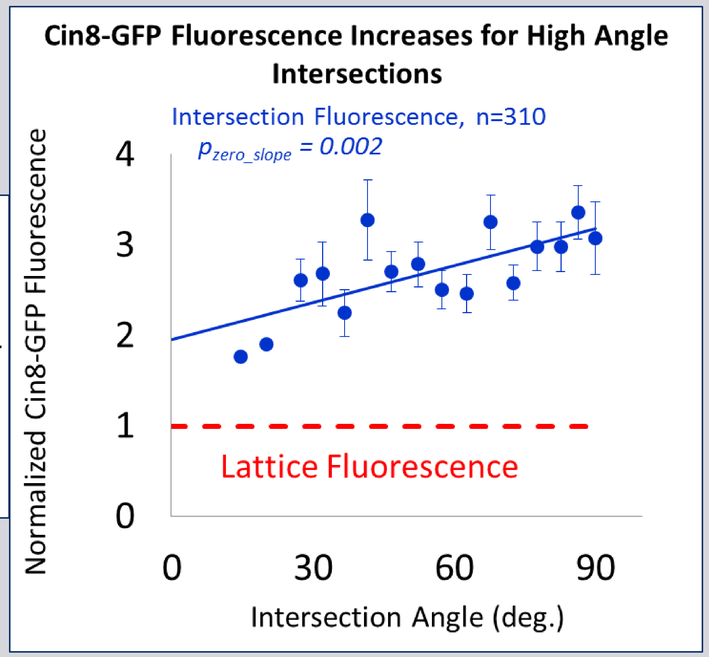



**Figure 2:** Experimental cartoon of the MATLAB code used to analyze the fluorescence of Cin8 on the microtubule lattice compared to the intersection.

**Figure 3:** Relationship between intersecting microtubule angle and normalized Cin8 fluorescence. This normalized fluorescence was found by the equation

$$\frac{I_{cross}}{I_{lat}} = normalized\ Cin8GFP\ Fluorescence.$$

In this equation, $I_{cross}$ represents the intensity of Cin8-GFP at the intersection, and $I_{lat}$ represents the intensity of Cin8-GFP at the lattice of the microtubule. The graph shows an increase of fluorescence as the angle between the two microtubules increases.



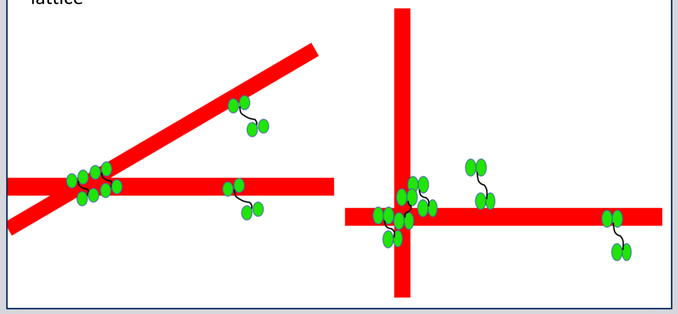

## RESULTS

- The results, shown as a graph in Figure 3, suggest a positive correlation between an increasing angle between the microtubules and the normalized Cin8-GFP fluorescence.
- As the angle between the microtubules increases toward 90 degrees, the normalized fluorescence of Cin8-GFP also increases, indicating that more Cin8 is present than at lower angles. This is shown in Figure 4.
- This correlation suggests that Cin8 does localize to an intersection compared to the lattice of the microtubule, seen by a lower lattice fluorescence in Figure 3.
- This figure also shows that any degree of intersection is at least a two-fold increase of fluorescence compared to the lattice.

**Figure 4:** Cartoon of results. The Kinesin-5 motor protein is shown as the green tetramer on red microtubules intersecting. As the angle between crossing microtubules increases, the amount of Cin8-GFP localized at the intersection also increases.

**Any crossing:**
Two-fold increase of GFP fluorescence compared to lattice

**90° cross:**
Three-fold increase of GFP fluorescence compared to lattice



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